MonoFas® DNA Purification Kit I
Operating Instructions

1 . Introduction

Thank you for purchasing the MonoFas® DNA Purification Kit IMonoFas® Spin Columns included in this MonoFas® DNA Purification Kit I are a filter type silica monolith consisting of continuous through-pores and silica skeletons. This column is a monolith solid phase column, being manufactured for DNA purification. There are 3 main advantages of using the MonoFas® DNA Purification Kit I.

1. Excellent liquid throughput. As the skeletons and DNA fragments can contact each other efficiently, it only takes about 4 minutes to purify the target DNA from the PCR products and 9 minutes from agarose gel.
2. There is no filter, hence high recovery and no damage of the sample. DNA fragments about 35 bp to 35,000 bp can be obtained.
3. High recovery rate of DNA fragments from the PCR reaction solution and agarose gel. High removal efficiency of primers, enzymes, substrates and buffers. There are no inorganic salts in the recovered DNA fragment solution.

More than 500 bases of the purified DNA fragments can be mapped with an accuracy of greater than 98% by a fluorescent sequencing method.

Read the following instructions before use to maintain good performance.

2 . Unpacking

- Check if anything is missing or damaged.

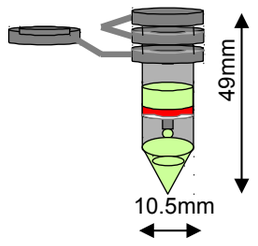
<CAUTION>

Due to the exporting regulation (IATA), ethanol canot be shipped. Please prepare ethanol before use to complete the Buffer B as described below.

Cat. No.	5010-21500	5010-21501	5010-21502
MonoFas® DNA Purification Kit	50 ea / pack	100 ea / pack	250 ea / pack
MonoFas® Spin Column	50 ea	100 ea	250 ea
Collection Tubes	50 ea	100 ea	250 ea
Buffer A (Binding, Dissolution)	30 mL x 1 ea	50 mL x 1 ea	50 mL x 3 ea
Buffer conc. B (Needs to be diluted to make	14mL x 1 ea Add anhydrous ethanol 26mL and	21 mL x 1 ea Add anhydrous ethanol 39mL and	21mL x 3 ea Add anhydrous ethanol 39mL and
Buffer C (Elution) 10 mM Tris-HCl 0.5mM EDTA	10 mL x 1 ea	10 mL x 1 ea	10 mL x 3 ea
Operating Instruction	1 ea	1 ea	1 ea

3 . How to Handle

- Do not drop or knock the MonoFas® Spin Columns or it may cause the monolithic silica gel to break.
- MonoFas® Spin Columns and collection tubes have already been sterilized.
- Autoclave within 20 minutes and do not exceed 110°C
- The guarantee period of unopened buffer solutions that are stored at room temperature is one year from the date of manufacture.
- Seal the cap tightly to prevent spilling of liquid.
- The diagram on the right is of a MonoFas® Spin Columnplaced in a provided collection tube. When using a centrifuge, make sure the sample tubes do not contact the lid of the centrifuge.



- MonoFas® Spin Columns are compatible with SPE accessories such as GL-SPE Vacuum Manifold and Qiavac6c (Luer adapter) vacuum manifold etc.
- Use well sterilized tubes and water to produce purified DNA for molecular biology operation.
- This product is disposable and cannot be reused.

4 . Specifications

	DNA purification from PCR reaction solution	DNA purification from agarose gel
Standard Operation Time	4 minutes	9 minutes
Maximum DNA Binding Capacity	10 μg	10 μg
Maximum Weight of Sliced Gel	—	<1g
Minimum Elution Volume	10 μL	10 μL
Column Reservoir Capacity	1mL	1mL
Available DNA Size	35～35,000 bp	35～35,000 bp
Recovery of DNA	>85% (100bp – 5kbp) >60% (5kbp – 35kbp)	>80% (100bp – 5kbp) >50% (5kbp –35kbp)
Primer Removal Rate	95%	-

- Recovery Rate for Each Elution Volume and DNA Sizes

Elution Vol (uL)	Recovery Rate (%)
5	83
10	89
15	91
20	88
25	83

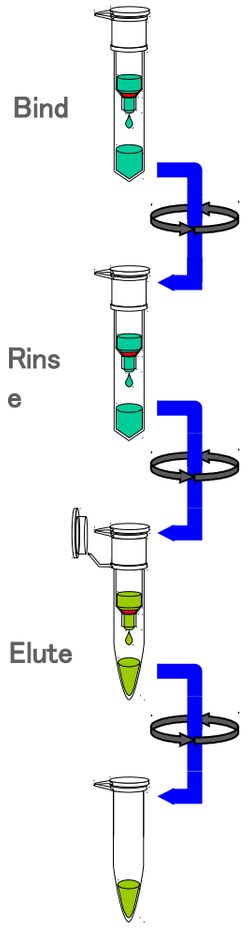
*400bp fragment DNA is used to measure the recovery rate for each elution volume.

Purified DNA can be used for …

Fluorescent Sequencing, Restriction Enzyme Digestion, Ligation, Cloning, Transformation
Northern Analysis, Southern Analysis, Labelled Reactant, Micro Injection, Micro Array Analysis
In Vivo Copy/Translation

5 . Protocol from PCR production

With this protocol, only the amplified product can be purified from the PCR reaction solution. Please set the centrifuge at 9,000 xg (10,000 rpm) before use.



1. Add Buffer A (10 times the sample volume) to the spin column (eg: 20ul sample: 200ul Buffer A)
2. Pour the sample into the spin column by pipetting a few times to spread. (Recommended Volume: 10-100ul)
3. Centrifuge (9,000xg, 30 seconds)
4. Pour in 500uL Buffer B.
5. Centrifuge (9,000xg, 30 seconds)
6. Switch the spin column to the micro centrifuge tube and add Buffer C
   (Recommended Volume: 10-50uL)
7. Centrifuge (9,000 xg, 1 minute)

DNA Size (bp)	Recovery Rate (%)
35	65
80	80
100	85
500	93
1000	96
2000	94
5000	92
10000	96
15000	96
20000	95
30000	92

<Cautions>

Step 1: **There is no need to dissolve the PCR products with Buffer A before adding to the spin column.** Leaving the Buffer A in the spin column for a long time may cause leakage.

Step 4: When treating more than 50uL sample, remove the solution in the collection tube after the step 4 and move to the rinsing step.

Step 6: Silica Monolith does not have any filter and provides an excellent liquid throughput, **hence there is no need for centrifuging after rinsing (step 6).**

Step 6: Buffer C consists of 10mM Tris-HCl+0.5mM EDTA (Free) (pH 8.5). It can be replaced with RNase/DNase free sterilized water. In this case, use ammonia water or aqueous potassium hydroxide to adjust the pH to 8.0-8.5, as the recovery changes depending on the pH.

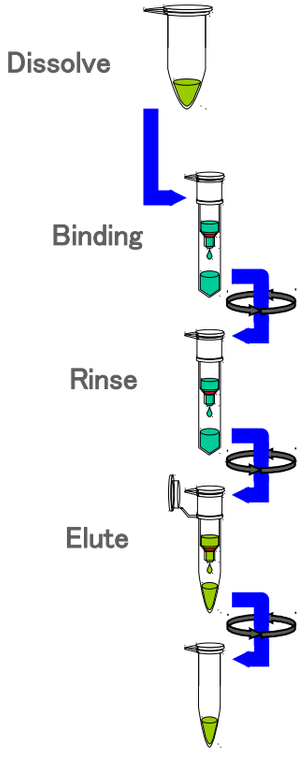
Step 3: Make sure that the gel is completely dissolved. If not, extend the heating time, raise the dissolving temperature, or stir during heating.

Step : **There is no need to centrifuge after rinsing (step 6).**

Step 8: Buffer C consists of 10mM Tris-HCl+0.5mM EDTA (Free) (pH 8.5). H_2O can be also used as the original elution solution. In this case, use ammonia water or aqueous potassium hydroxide to adjust the pH to 7.0-8.5 as the recovery changes depending on the pH.

6 . Protocol from Agarose Gel

With this protocol, only DNA fragments can be purified from agarose gel. Please set the centrifuge at 9,000 xg (10,000 rpm) and a heat block or water bath heated at 60 ℃ before use.



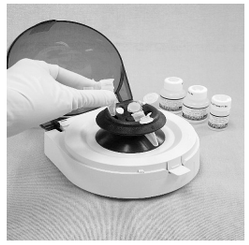
1. Take the target band from the agarose gel and slice at a clean place.
2. Weigh the gel and put the same amount of Buffer A as the gel into the micro tube (eg: Gel 50mg: Buffer A 50ul)
3. Keep it at 60 °C for 5 minutes (to dissolve sample)
4. Pipette the dissolved sample onto the spin column.
5. Centrifuge (9,000xg, 30 seconds)
6. Add Buffer B 500uL.
7. Centrifuge (9,000xg, 30 seconds)
8. Switch the spin column to the micro centrifuge tube and add Buffer C.
9. Centrifuge (9,000xg, 1 minute)

<Cautions>

Step 1: Make sure that the gel is to be cut by a clean cutter or knife, and remove the unnecessary water.

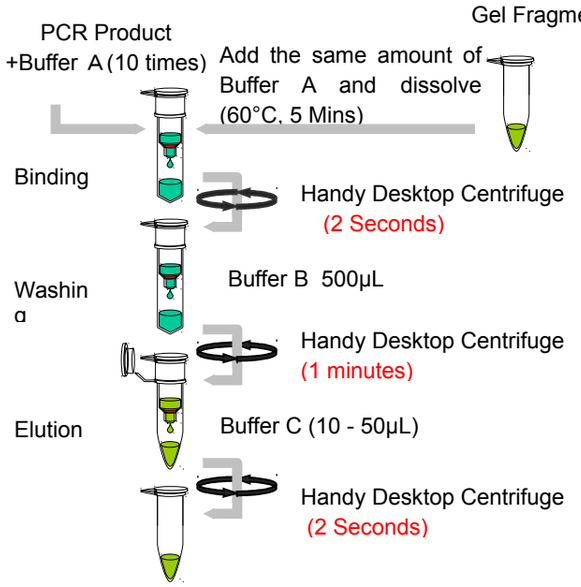
7 . Protocol using a Centrifuge

The MonoFas® DNA Kit I can be used with the handy desktop centrifuge. This protocol can be used for both purifications from PCR products and agarose gel. With the handy desktop centrifuge, the purification from PCR products takes only 2 minutes and 7 minutes from agarose gel.



Handy Desktop Centrifuge Machine

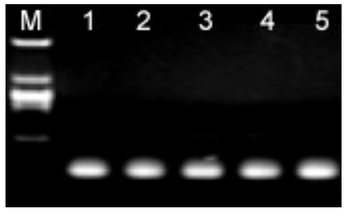
Number of Cycles
More than 6,200 rpm
±20%(Fixed)
Centrifuge Acceleration
More than 2,000 *xg*
(19,600 m/s^2)



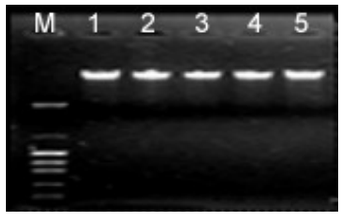
Recovery does not change depending on the number of revolutions and revolution

○ Recovery change by centrifuge method and time

●Fragment DNA :35 bp



●Fragment DNA :35 kbp



Lane #	Items	Binding		Rinsing		Elution	
		# of cycles (rpm)	Centrifuge Time	# of cycles (rpm)	Centrifuge Time	# of cycles (rpm)	Centrifuge Time
M	pHY Marker	-	-	-	-	-	-
1	Unpurified PCR products	-	-	-	-	-	-
2	Existing Centrifuge	10,00	30secs	10,000	1min	10,000	30secs
3	Existing Centrifuge	5,000	30secs	5,000	1min	5,000	30secs
4	Handy Desktop		2secs		1min		2secs
5	Handy Desktop		5mins		5 mins		5mins

Fragment sizes between 35 bp and 35 kbp can be purified by spinning down.
Please make sure to have centrifuge for 1 minute after the rinsing step.

8. Troubleshooting

Trouble	Possible cause	Solutions
Low or no recovery	Inadequate amount of Buffer A was added to the sliced gel and PCR reaction solution.	Check the volume of buffer added to the sliced gel and PCR reaction solution. Add 10µL of Buffer A for every 10mg of sliced gel. Add 100µL of Buffer A for every 10µL PCR reaction solution.
	The original elution buffer was not appliedproperly.	Make sure that the original elution buffer was pH 8.0-8.5.
	Elution Buffer C is not diffusing on the surface of the monolith.	Add Buffer C to the central part of the monolithic silica surface.
	The gel is not completely dissolved.	Check if the gel has been completely dissolved. Extend the dissolving time. During the dissolving, stir the sample a few times.
Cannot obtain a good result in fluorescent sequencing	The amount of DNA for sequencing reaction is too little.	Increase the amount of DNA for sequencing reaction. Concentrate DNA by ethanol.
	The amount of DNA for sequencing reaction is too much.	Dilute the amount of DNA for sequencing reaction.
The restriction enzyme digestion is not working.	Inadequate enzyme concentration and digestion time.	Increase the amount of the enzyme and the digestion time. Digest it at the adequate temperature and use an optimal buffer.
	The DNA that passed through the column includes ethanol and salt.	Precipitate the DNA by using ethanol. Set the volume of DNA to 10% of the total reaction volume.
The purified DNA is less in quantity compared to the DNA that was confirmed by the agarose gel.	Contaminants exist in the agarose gel DNA.	Quantitate the amount of DNA on the agarose gel by using ethidium bromide.
	Contaminants exist in the recovered DNA from the column.	Re-purify the eluted DNA by using the spin column. Set the volume of DNA to 10% of the total reaction volume.
The absorbance ratio A260/280 of the purified DNA is low.	Some Buffer A remaining Inadequate cleaning with Buffer B Some Buffer B remaining Inadequate centrifugal rotations or time Inadequate vacuuming pressure	Precipitate the DNA by using ethanol and recover it. If necessary, purify it again with the monolith column. More than 20 in Hg of vacuuming pressure is needed in the suction method. If the vacuuming is inadequate, use the centrifugation method.
Cannot apply the purified DNA to the agarose gel.	Some Buffer B remaining	Re-purify the eluted DNA by using the spin column. Double up the amount of the Binding Buffer.
Cannot confirm or identify the band of the purified DNA.	DNA has been sheared. Agarose gel was not dissolved enough.	Mix agarose gel and Buffer A well.
	DNA was decomposed by nuclease.	Confirm the guarantee period of each buffer solution. Sterilize them again.
Cloning efficiency is low.	Buffer A is still remaining. Buffer B is still remaining. Agarose gel is mixed.	Check each step and protocol and make sure to follow them.
	Primer-dimer was spiked in the eluting solution.	A primer-dimer bigger than 20bp was formed and was not removed completely. After the adsorption step, use 35% guanidine hydrochloride solution to rinse. Then use Rinsing Buffer B and move to the Elution step.
	Eluate contains ssDNA	Prepare a reaction solution set the temperature at 95℃ and incubate for 2 minutes. Cool it down gradually. Then add enzyme to oxidize.
	Smears can be seen by gel electrophoresis	Salt might be contained in the eluate solution

9 . Storing of the kit

- Store the MonoFas® DNA Purification Kit lin a clean place with a constant temperature.
- The guarantee periods of each unopened buffer solution being stored at room temperature is one year from the manufacturing date.

10 . Warranty

The MonoFas® DNA Kit lis manufactured, inspected, packaged and shipped under our strict standards of quality control. Should you find any defect in performance, please contact us.

This product is manufactured for the purpose of DNA purification only.

We regret that we cannot accept any claim when their performance has deteriorated due to non-compliance with the above operating instructions.

The guarantee periods of each unopened buffer solution being stored at room temperature is one year from the manufacturing date.

11 . Product Information

Products	Cat. No.	Volume
MonoFas® DNA Purification Kit lBuffer A	5010-21506	50 mL
MonoFas® DNA Purification Kit lBuffer B	5010-21507	60 mL
MonoFas® DNA Purification Kit lBuffer C	5010-21508	10 mL

Products	Contents	Cat. No.	Volume
MonoFas DNA Purification KitI	Fragment DNA Purification Kit	5010-21500	50pcs
		5010-21501	100pcs
		5010-21502	250pcs
MonoFas DNA Pufirication Kit II	Genomic DNA Purification Kit from Whole Blood	5010-21511	96pcs
	Adaptor for Micro Tube Centrifuge	5010-21513	6pcs
MonoFas DNA Purification Kit I II	Plasmid Extraction Kit	5010-21503	50pcs
		5010-21504	100pcs
		5010-21505	250pcs
MonoTip C18	Desalting / Enrichment of Protein	5010-21001	24pcs
		5010-21000	96pcs
MonoTip mini C18	Desalting / Enrichment of Small amount of Protein	5010-21202	24pcs
		5010-21200	96pcs
MonoTip Trypsin	Trypsin Digestion of Protein	5010-21012	24pcs
		5010-21010	96pcs
MonoTip TiO	Selective Enrichment of Phosphopeptide	5010-21007	24pcs
		5010-21005	96pcs

"Based on monolithic technology, Merck KGaA, Darmstadt, Germany"

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